Verbenone, Conophthorin, and Methyl Jasmonate:
Protecting Spruce from Attack by *Ips perturbatus* (Scolytidae) in Alaska

Update on 2004-2005 FHTET-funded project from the Bark Beetle Technical Working Group Meeting (Homer, Alaska, 12-14 October, 2004)

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Background

The Northern spruce engraver, *Ips perturbatus* (Eichhoff) (Coleoptera: Scolytidae), is a bark beetle that generally colonizes and kills white spruce, *Picea glauca*, and Lutz spruce, *Picea xlutzii* (Moench) Voss (Fig. 1). *Ips perturbatus* has an extensive boreal distribution that includes most of Canada and parts of the northern U.S., including: Alaska, Montana, Minnesota, Michigan, and Maine (Bright 1976; Wood, 1982).

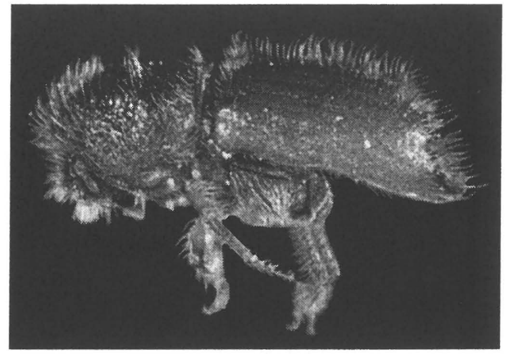

Figure 1. The Northern spruce engraver, *Ips perturbatus* (Eichhoff) (Coleoptera: Scolytidae).

Recent research on the management of *I. perturbatus* involves attractive and interruptive behavioral chemicals to trap or disrupt populations (Holsten *et al.*, 2000) (Fig. 2, Table 1). The combination of verbenone and conophthorin significantly reduces the number of *I. perturbatus* responding to attractant-baited Lindgren funnel traps (Graves *et al.* unpublished data).

A promising new technology for enhancing the resistance of conifers to bark beetles and fungi is the application of the naturally occurring, and commercially available, plant hormone jasmonic acid or its analogue methyl jasmonate (MJ) (Fig. 3) (Creelman and Mullet, 1997). These compounds have been shown to cause anatomical changes in Norway spruce, *Picea abies*, Douglas-fir, *Pseudotsuga menziesii*, western white pine, *Pinus monticola*, and many other conifers, related to oleoresin and phenolic synthesis and storage (Franchesci *et al.*, 2002; Martin *et al.*, 2002; Hudgins *et al.*, 2003, 2004; Hudgins and Franchesci, 2004; Huber *et al.*, 2004). In spruce and Douglas-fir, MJ induces accumulation of resin chemicals, gene expression, and enzyme activities of metabolic pathways related to terpene synthesis (Martin *et al.*, 2002; Fäldt *et al.*, 2003; Huber *et al.*, 2004). Specifically, it increases the activity of enzymes called monoterpene synthases directly responsible for the accumulation of monoterpenes in the oleoresin. The purpose of this document is to summarize two years of study on the effects of verbenone, conophthorin, and MJ on the response and attack behavior of *I. perturbatus* on healthy *P. xlutzii* in Alaska.

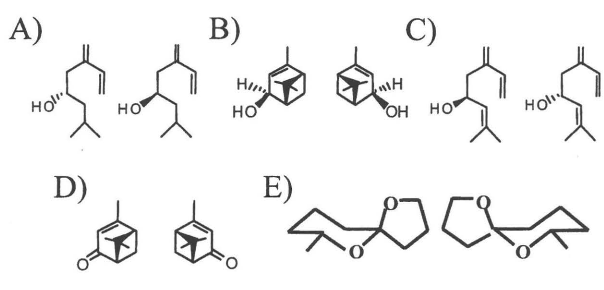

Figure 2. Chemical structures of behavioral chemicals associated with recent research on *Ips perturbatus* including the three components of the *I. perturbatus* attractant: A) ipsenol, B) *cis*-verbenol, C) ipsdienol, and two interruptive compounds: D) verbenone, and E) conophthorin.

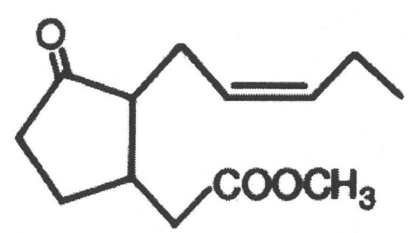

Figure 3. Chemical structure of MJ ($C_{13}H_{20}O_3$, molecular weight = 224.3)

2004 Field Research

Location. Near Silvertip Creek, approx. 150 km south of Anchorage on the Kenai Peninsula, AK in a naturally regenerated stand of Lutz spruce, *P. xlutzii* (N 60° 44' W 149° 21').

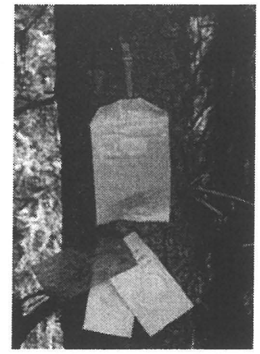

Figure 4. Semiochemical treatment stapled to the south-facing side of an attractant + verbenone + conophthorin-treated tree.

Objectives. **1)** Assess the attack behavior of *I. perturbatus* in response to attractant-baited trees with and without verbenone and conophthorin. **2)** Evaluate the effects of sodium *N*-methyl-dithiocarbamate (MS) (a soil fumigant) on tree health.

Methods. Treatments were applied to trees on 25 May 2004. There were five treatments in the study (Table 2); each treatment was replicated ten times; and the study was arranged in a completely randomized design. Fifty trees were selected (>12 cm diameter at breast height) with a distance of at least 30 m between selected trees. Following tree selection, treatments were assigned and all semiochemical release devices were stapled to the south-facing sides of trees on 25 May 2004 (Fig. 4). Attack density was assessed at 1.6 m height on 3 August 2004; mortality was assessed on 3 August 2004 and again on 7 June 2005.

Statistical Analyses. Attack density data was analyzed using the one-way ANOVA procedure and the means were compared using REGWQ (Day and Quinn, 1989). Tree mortality data (expressed as a percentage of ten trees) was analyzed using the Chi-square procedure.

Results.

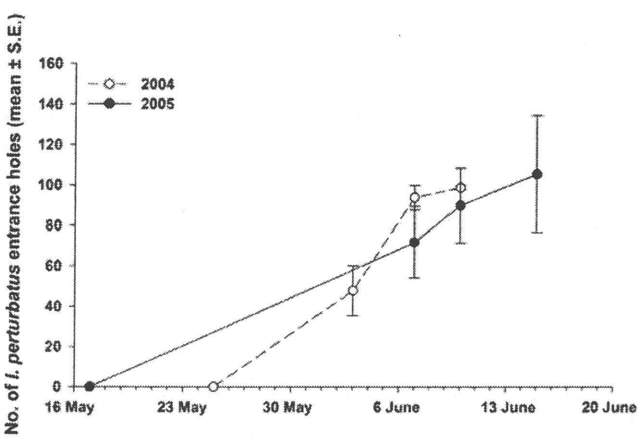

Figure 5. Attack progression by *Ips perturbatus* on attractant-baited trees at Silvertip, Kenai Peninsula, AK (N=10). Data were extrapolated from four samples per tree (each 100 cm^2) to estimate the number of *I. perturbatus* entrance holes from the base to approx. 1.75 m on the trunk of the tree.

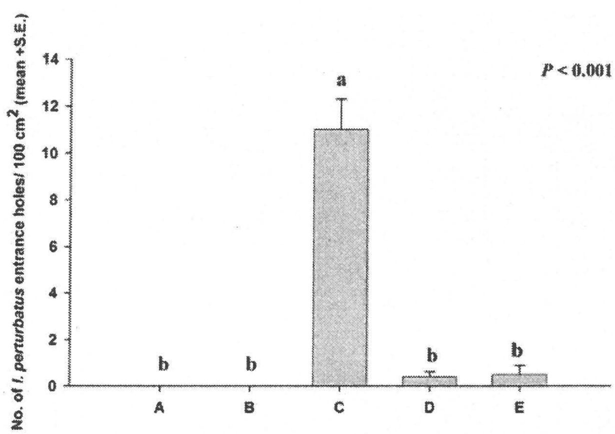

Figure 6. *Ips perturbatus* attack density on standing *Picea xlutzii* trees at Silvertip, Kenai Peninsula, AK (3 August 2004) (N=10). Treatments are as follows: A) untreated, B) verbenone and conophthorin, C) *I. perturbatus* attractant (synthetic ipsenol, ipsdienol, and *cis*-verbenol, D) attractant + verbenone +conophthorin, E) injected MS in DMSO.

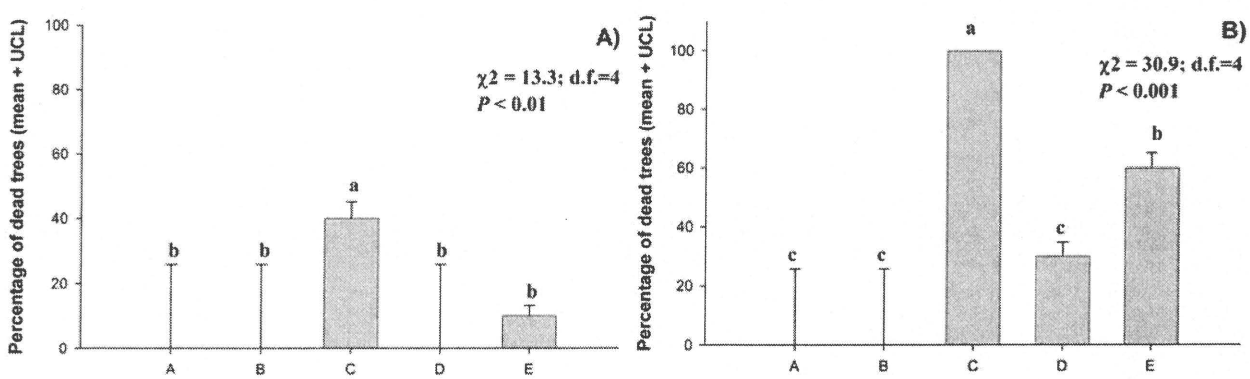

Figure 7. Mortality of *Picea xlutzii* attributed to *Ips perturbatus* at Silvertip, Kenai Peninsula, AK, A) 3 August 2004 and B) 7 June 2005 (N=10). Treatments are as follows: A) untreated, B) verbenone and conophthorin, C) *I. perturbatus* attractant (synthetic ipsenol, ipsdienol, and *cis*-verbenol, D) attractant + verbenone +conophthorin, E) injected MS in DMSO.

2005 Field Research

Location. Same as 2004.

Objective. Assess the attack behavior of *I. perturbatus* in response to attractant-baited trees with and without verbenone, conophthorin, MJ spray, or MJ injection.

Methods. There were 11 treatments in the study (Table 2). Each treatment was replicated ten times, and the study was arranged in a completely randomized design. One hundred and ten trees were selected (>12 cm diameter at breast height) with a distance of at least 30 m between selected trees. Two liters of MJ [100 mM in aqueous Tween 20 (0.1%)] or Tween 20 in water were applied evenly from the base to a height of 3.7 m to each relevant experimental tree (Fig. 8). The first application was on 18 May and the second application was on 1 June, 2005. Methyl jasmonate was also formulated at a much higher concentration for injection in a second set of experimental trees. Next, this solution was injected into 12 holes per tree (four ml per hole) using a 10 ml plastic syringe (Fig. 9). The first set of injections was performed on 19 May 2005, whereas the second set was performed on 2 and 3 June 2005. Concurrently with the MJ injections, a solution of Tween-20 was injected identically into another set of control trees. Following application of the spray and injection treatments, semiochemical treatments were applied on 3 June 2005 in the same manner as in 2004.

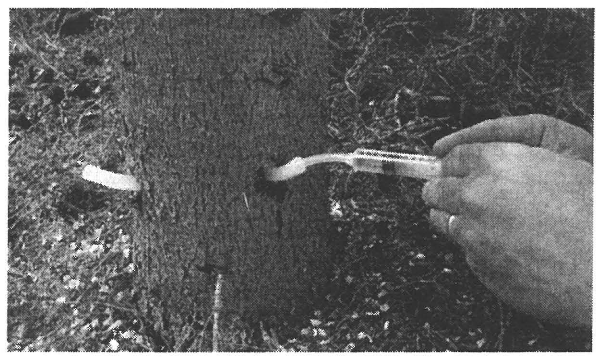

Figure 8. Spray application of MJ in Tween 20 to a standing *Picea xlutzii* tree at Silvertip, Kenai Peninsula, AK,

Statistical Analyses. Same as above.

Results.

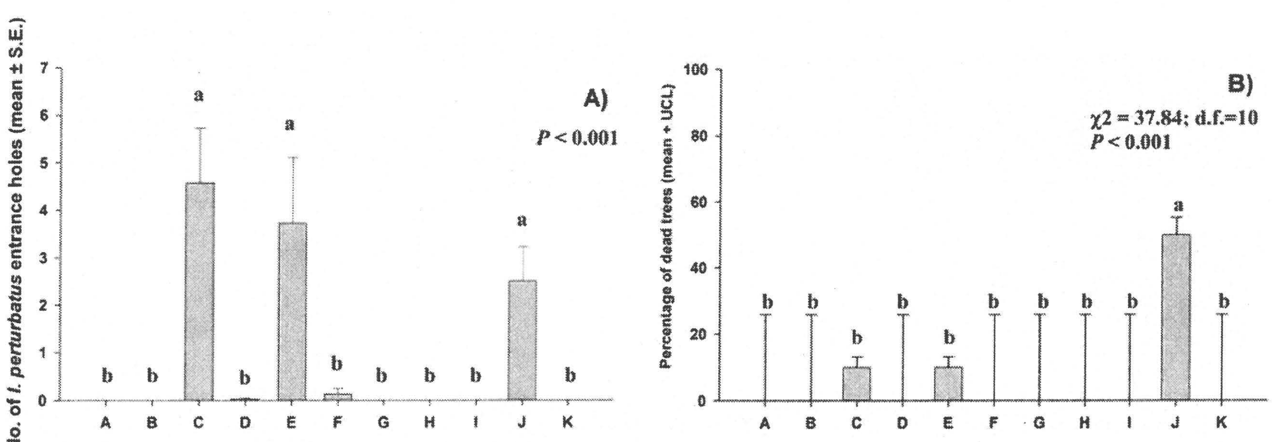

Figure 10. A) *Ips perturbatus* attack density on standing *Picea xlutzii* trees at Silvertip, Kenai Peninsula, AK (16 August 2005) (*N*=10) and B) Observed mortality of *Picea xlutzii* attributed to *I. perturbatus* at Silvertip, Kenai Peninsula, AK (18 August 2005) (*N*=10). Treatments include: A) untreated, B) Tween sprayed, C) *I. perturbatus* attractant (synthetic ipsenol, ipsdienol, and *cis*-verbenol, D) attractant + verbenone + conophthorin, E) attractant + methyl jasmonate (MJ) spray, F) attractant + MJ spray + verbenone + conophthorin, G) MJ spray, H) verbenone + conophthorin, I) MJ + verbenone + conophthorin, J) attractant + MJ injection, K) Tween injection.

Conclusions.

- Treatment with a simple, two-component interruptant system of verbenone and conophthorin significantly reduced attack density on attractant-baited trees in two studies and has provided a full year of protection in one study.
- Regardless of treatment, trees treated with verbenone and conophthorin had significantly lower attack densities than any treatment containing the attractant.
- Treatment with MJ (spray or injection) did not significantly reduce attack density on attracted-baited trees in one study.
- Treatment with verbenone and conophthorin and/or MJ does not attract beetles to uninfested trees.
- Treatment of attractant-baited trees with verbenone and conophthorin significantly reduced mortality at two assessment times (3.5 and 12.5 mos. post-treatment) in one study.
- The rate of mortality of verbenone and conophthorin-treated trees was not significantly different from the rate of mortality in unbaited trees in two studies.

TABLE 1. RELEASE RATES AND CHEMICAL PURITIES OF SYNTHETIC SEMIOCHEMICALS USED IN *IPS PERTURBATUS* TRAPPING AND STANDING TREE STUDIES AT SILVERTIP CREEK, KENAI PENINSULA, ALASKA, 2004-2005

Semiochemical	Enantiomeric composition	Load (mg)[1]	Release rate (mg/day)[2]	Chemical Purity	Cost/Device[3]
Ipsdienol	Racemic	40	0.2	> 98%	$ 2.96
Ipsenol	Racemic	20	0.2	> 98%	$ 4.48
cis-Verbenol	83%-(–)	75	0.3-0.6	> 98%	$ 3.16
Commercial Verbenone (pouch)	84%-(–)	4.7[4]	40.0	> 97%	$ 6.28
(*E*)-conophthorin	Racemic	70	3.0	~90%	$13.68
Methyl jasmonate	n.a.	100	??	95%	$ 0.25
Methyl jasmonate	n.a	1.0[4]	50-60	95%	$ 3.00

[1] Polyethylene centrifuge tube release device (400 μl) for conophthorin and methyl jasmonate (low release rate), urethane pouch for verbenone and methyl jasmonate (high release rate); all other semiochemicals used a polyvinyl chloride bubble cap release device.

[2] Release rates determined at 22^{0} C.

[3] 2005 Cost Estimates

[4] Release device load in grams.

TABLE 2. TREATMENTS USED IN EXPERIMENTS IN 2004 AND 2005 STANDING TREE STUDIES AT SILVERTIP CREEK, KENAI PENINSULA, ALASKA

2004	2005
Unbaited	Unbaited tree
Unbaited verbenone pouch (±)-*trans*-conophthorin	Tween sprayed Attractant-baited
Racemic ipsenol, racemic ipsdienol 83%-(–)-*cis*-verbenol (Attractant)	Attractant-baited, verbenone, conophthorin
Attractant-baited verbenone pouch (±)-*trans*-conophthorin	Attractant-baited, MJ-sprayed
Artificially-stressed tree injected MS dissolved in DMSO	Attractant-baited MJ-sprayed, verbenone, conophthorin
	MJ-sprayed
	Verbenone, conophthorin
	MJ-sprayed verbenone, conophthorin
	Attractant-baited, MJ-injected
	Tween-injected

Acknowledgements. We thank Ed Holsten, John Hard, and Ken Zogas (Forest Health Protection, Anchorage) for assistance with this study. We also thank Region 10 Forest Health Protection and Harold Thistle (FHTET, Morgantown) for financial assistance. Finally, we thank David Wakarchuk (Synergy Semiochemicals, Corp, Burnaby) for advice on formulation of MJ for application to trees and as a trap bait.